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(54) Title: METHOD OF LIQUID STERILIZATION

(57) Abstract

A method is provided for sterilizing medical devices using a solution of polyepoxy compounds, particularly glycidyl ethers such as those registered under the trademark name Denacol®. These polyepoxy compounds are especially effective at concentrations as low as 2 to 4 volume percent in aqueous solution for sterilizing biologic medical devices, such as tissue heart valves or vascular grafts, without causing damage to the tissue of the implant and without leaving a residue on the device that is toxic to the tissue of the patient.

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METHOD OF LIQUID STERILIZATION

This invention relates to a method of sterilizing medical devices using liquid sterilants. More particularly, this invention relates to a method of sterilizing implantable biological devices using polyepoxy solutions.

Medical devices are routinely sterilized to reduce to a minimum the population of microbial life found on their surfaces. The measurement standard used by industry for evaluating the effectiveness of a sterilization procedure is known as the Sterility Assurance Level (SAL). The level of SAL acceptable by the medical industry is 6, which represents the probability that a surviving target organism remains after the lapse of a specified time period is less than 1×10^{-6}. The amount of time necessary to achieve the acceptable SAL for a particular sterilization procedure as used on a specific target organism is calculated from a measured unit of time known as the D-value.

The D-value generally represents the amount of time necessary to achieve a single ninety (90) percent reduction in the count level of viable target organisms after the application of the sterilization procedure, with a lower D-value representing a more efficient procedure. The D-value time also depends upon the conditions under which the procedure is performed, such as the temperature and the specific sterilant being used. Sterilization can be accomplished by subjecting the device to a variety of means for denaturing the microbes, such as ultraviolet radiation, extreme heat, and a variety of chemical disinfectants. Many of the chemical sterilants are liquids or are used in liquid solutions.

Unlike the other means of sterilizing medical devices, the use of chemical sterilants almost inevitably leaves a

residue on the surface of the device. This residue of chemical sterilant is often toxic and/or in some way harmful to the host tissue brought into contact with it. This biological response is expected due to the inherent antimicrobial properties of the sterilants. To negate this potential host response to the sterilant residuals, medical devices are routinely exposed to various rinsing procedures prior to implantation to reduce these sterilant residuals to a non-toxic level.

Implantable biological devices, valves and venous grafts, are made from various types of animal tissue, such as bovine and porcine. Such devices are destroyed by high heat and ultraviolet sterilization techniques. Therefore, chemical sterilization is necessary. Generally, these implantable biological devices have already been fixed using chemicals such as glutaraldehyde, formaldehyde, hexamethylene diisocyanate or polyepoxy as a fixative to cross-link the collagen and thereby prevent calcification of the graft in situ. It is important in selecting a sterilizing chemical for a biological device, therefore, that it not adversely interfere or react with any residue of the fixative remaining on the fixed prosthetic device. U. S. Patent 4,806,595 and pending U. S. patent application Serial No. 253,347, filed September 9, 1988 discuss various methods of fixing biological tissues using chemical fixatives.

Generally, biological prosthetic devices are packaged and stored submerged in the liquid sterilizing solution to maintain their natural flexibility and to inhibit microbial proliferation during the storage period.

Biological devices are implanted within living bodies, usually to replace a diseased or damaged natural part that has been surgically removed. It is also particularly important, therefore, that the sterilant used be capable of

reducing and suppressing to an acceptably low level the population of microbes on the device without leaving a chemical residue that is toxic or inhibits infiltration of host cells into the implanted device.

Microbial viability depends upon the action of certain essential enzyme systems. When these enzyme systems are interrupted, the result is an irreversible change in specialized proteins that are necessary to continuation of the microbial life cycle. It is particularly desirable, therefore, to find a chemical sterilant effective at relatively low temperatures that interferes with the enzyme systems necessary to the microbial life cycle.

SUMMARY OF THE INVENTION

A method is presented for sterilizing a medical device comprising contacting the device with an effective amount of at least one polyepoxy compound so as to substantially reduce the microbial population thereon. Preferably an aqueous solution containing from 2 to 4 volume percent of a glycidyl ether is used to sterilize a medical device such as a biological implantable device.

DESCRIPTION OF THE DRAWING

Figure 1 is a log graph illustrating the number of surviving Bacillus pumilus with time upon exposure to various sterilizing solutions containing 2% by volume of ethylene glycol diglycidyl ether.

Figure 2 is a log graph illustrating the number of surviving Bacillus pumilus with time upon exposure to various sterilizing solutions containing 2% by volume of ethylene glycol diglycidyl ether.

Figure 3 is a log graph comparing the number of surviving Bacillus pumilus upon exposure at various temperatures to sterilizing solutions containing either 2% or 4% by volume of ethylene glycol diglycidyl ether.

DETAILED DESCRIPTION OF THE EMBODIMENTS

In accordance with the present invention, it is contemplated that various types of implantable biological tissue derived from numerous animal sources and parts of the anatomy can be sterilized with less danger of toxic residue than with conventional sterilization procedures. The tissue can be derived from various sources, including but not limited to, bovine, porcine, horse, sheep, kangaroo, and rabbit. All kinds of implantable tissue, such as tendons, ligaments, heart valves and tissue used to construct heart valves, such as dura mater and pericardium, as well as tissue used for augmentation, such as skin patches, pericardial patches, aortic patches, and tympanic membranes are suitable for sterilization according to the present invention. Moreover it is contemplated that the surfaces of non-biological items such as surgical instruments, medical devices, valve conduits and the like made of plastic, cloth, or metal, can be effectively treated according to the present invention.

It has been discovered that polyepoxy compounds, particularly glycidyl ethers such as those registered under the trademark name Denacol® (manufactured by Nagase Chemical Ltd, Hyogo-ken, Japan) are particularly effective and liquid sterilants for use in sterilizing medical devices. Polyepoxy compounds have epoxy functional groups

$$CH_2 - CH-$$
$$O$$

that react very easily with amino, carboxyl, and hydroxyl groups in proteins to cross-link them. The cross-linking of proteins in microbes makes their germination and reproduction processes impossible, thus effectively killing them off.

Glycidyl ethers, especially diglycidyl ethers having the following general formula:

$$CH_2\text{-}CH\text{-}CH_2\text{-}O\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}CH_2\text{-}CH\text{-}CH_2$$
$$\qquad\qquad O \qquad\qquad\qquad\qquad\qquad\qquad O$$

wherein *n* is an an integer from 1 to about 6 are preferred.

Nonlimiting examples of the polyepoxy ethers that can be used as liquid sterilants for medical devices are glycerol polyglycidyl ether, trimethylol polyglycidyl ether, diglycerol polyglycidyl ether, polyglycerol polyglycidyl ether, ethylene and polyethylene glycol diglycidyl ether, propylene and polypropylene glycol diglycidyl either, neopentyl glycol diglycidyl ether and 1,6-hexanediol diglycidyl ether, among others. Of these, four types are preferred: diglycidyl ether of glycol, polyglycidyl ether of polyol, diglycidyl ester of dicarboxylic acid and monoglycidyl ether of polyol. These compounds are especially preferred for sterilizing biological devices that have been fixed in a polyepoxy fixative as disclosed in U.S. patent 4,806,595 mention above, which is hereby incorporated by reference in its entirety.

For economy, ease of manufacture, and to avoid irritation to patients contacted with sterilized medical devices, the epoxy compounds used as sterilants are generally used in an aqueous solution or formulation at a concentration of less than about 10 volume percent. Preferably, however the concentration of the polyepoxy compound in the sterilizing solution is no more than from about 2 to 4 volume percent. Although the polyepoxy compounds used in the practice of this invention are known to be systemically toxic as well as intracutaneously irritating and mutagenic, at the low concentrations

required to effectively sterilize medical instruments as taught herein, the residual sterilant remaining on the devices has been found to have substantially no toxic effects on living matter.

Since epoxy compounds are not very soluble in water, a sufficient amount of a surfactant or a biocompatible surface tension-reducing compound, such as an alcohol having from 1 to about 5 carbon atoms, can be used to reduce the surface tension and promote dissolution of the polyepoxy compound in water. For sterilizing biological medical devices, care must be taken as well that the surface tension-reducing agent is not harmful to the tissue in the device.

Suitable alcohols include biocompatible aliphatic and aromatic alcohols, preferably aliphatic alcohols containing from 1 to about 5 carbon atoms. Aliphatic alcohols include but are not limited to methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, sec-butanol, t-butanol, cyclo-hexanol, n-octanol, allyl alcohol, and the like. Suitable biocompatible aromatic alcohols include benzyl alcohol, cresol, carbinol, and the like. These alcohols are generally effective in themselves in destroying microorganisms and thus cooperate with and enhance the sterilizing capacity of the polyepoxy compounds. However, in the treatment of biological tissue devices, the upper end of the range of their usefulness must be limited by the impact or effect of the alcohol on the integrity of the tissue, and may depend somewhat on the type of alcohol used. For instance, no more than about 22 volume percent of ethanol can be used in the sterilization of biological devices without causing damage to the tissue of the device.

Suitable organic surfactants include anionic, cationic, and nonionic surfactants and their salts. The preferred

salts of the surfactants in the present invention include sodium and potassium. Anionic surfactants useful in the present invention are those having a relatively large hydrophobic region of hydrocarbon residues, including aliphatic and aromatic groups, bonded to a negatively charged ionic group. The aliphatic residues may be branched chained, straight chained, cyclic, heterocyclic, saturated, or unsaturated. These hydrophobic residues can be connected directly to an anionic group, such as carboxylate, sulfate, or sulfonate; or can be connected thereto through an intermediate linkage, such as an ester, amide, sulfonamide, ether, or aryl group. Anionic surfactants in one embodiment of the present invention are those having one or more carboxylates bonded to an alkyl side chain, to a steroid, or to amino acids in the side chain, such as in the bile acids.

Generally, from about 3 to 30, and preferably from about 5 to 15 volume percent of the surface tension reducing compounds is added to an aqueous solution. The most preferred surface reducing compounds are selected from the group consisting of ethanol and isopropanol.

In use, the medical device is submerged in the liquid sterilant or liquid sterilant solution under conditions of time and temperature selected to assure that at least about 90 percent of the microbial life present on the medical device will be killed. The percent kill can usually be increased just by increasing the temperature of the solution and/or extending the sterilization time. Generally the temperature of the solution is maintained in the range from room temperature to about 100 degrees C. for surgical instruments or medical devices in general, but for biological devices the preferred temperature range is from about 20 (room temperature) to about 45 degrees C. to avoid damage to the tissue.

The optimum sterilization time is related to the quantity of microorganism present and the level of sterility assurance desired. Consequently, the time can be varied according to needs. The interplay between time and temperature illustrated in the examples hereinafter dictates that if the temperature is increased, the time of exposure needed to achieve any selected level of sterility would necessarily decrease. Accordingly, if the temperature is decreased, the exposure time would have to be increased significantly.

Generally, however, the sterilization time needed to reduce the population of microorganism by about 90 percent ranges from about 5 to 120 hours, preferably 4 to 60 hours. If the device is packaged and stored in the solution, of course, sterilization can continue throughout the shelf life of the packaged device.

In accordance with the present invention, a sterilant composition is considered potentially effective if it shows about a 90% reduction of the organisms being tested. Preferably an effective solution shows a complete destruction of a large quantity (10^5 to 10^6) of test organisms within an acceptable time frame, usually from about 7 to about 8 hours). It is known that in many cases a microorganism in solution is easier to kill than one located on a surface or substrate. Therefore, the sterilant compositions herein are considered particularly effect if the organism destroyed is on a surface rather than in solution or on tissue.

The following examples and the other disclosure of this application are provided for illustrative purposes only, and are not intended to limit the scope of the invention, which is as described in the claims below.

EXAMPLE 1

Microbiological Testing

A variety of sterilant solutions were evaluated using various concentrations of sterilant, combinations of additives and conditions of time and temperature to determine the efficiency of the sterilants for killing microbes. In vitro tests were conducted by placing a known concentration of Bacillus pumilus or Microascus cinereus into 100 ml. of the sterilant solution. At designated, timed intervals, aliquot samples were withdrawn and immediately processed by a filtration/rinsing procedure utilizing 0.45 micron membrane filter and USP Fluid A rinse to remove sterilant residue. The survivors on the membrane filters were cultured on Tryptic Soy Agar, by incubating at 30-35 degrees C. for 14 days before a count of survivors was taken. The microbes used were Bacillus pumilus, American Type Culture Collection #27142, at an initial concentration of 10^3 spores per ml. of test solution and Microascus cinereus, American Edwards Laboratory #80-076, at an initial concentration of 10^4 spores per ml. of test solution.

The sterilant used was ethylene glycol diglycidyl ether (Denacol® EX 810) in aqueous solution to which as surface tension reducing agents were added 200 proof ethanol (E), 0.1 volume percent of 2,4,6-tris-(dimethylaminomethyl)phenol (DMP - 30), and 0.007 volume percent of salicylic acid in distilled water in concentrations shown in Table 1 below. The pH of the sterilization solution was adjusted to 9.5-10.0 with 1N HCl. D_{10} values (liquid sterilant exposure time at temperature needed to affect a 90% reduction in the microbial population) for the sterilant solutions were calculated utilizing Least Square Analysis methodology. A summary of the results appears in Table 1 below.

TABLE 1

CALCULATED BACILLUS PUMILUS AND MICROASCUS CINEREUS D_{10} VALUES WITH VARIOUS DENACOL® SOLUTIONS AND TEMPERATURES

Denacol[a] solution %(v/v)	M.cinereus 37°C (hr)	Bacillus pumilus RT (hr)	37°C (hr)	45°C (hr)
2%D+5%E[a]	---	34.3	---	---
2%D+5%E	---	38.4	12.3	---
2%D+5%E+buffer[b]	---	72.5	23.6	---
2%D+10%E	---	38.5	7.56	---
2%D+20%E	0.37	28.9	7.0	3.8
2%D+20%E+0.1%T[c]	---	37.1	---	---
4%D+5%E	---	16.18	2.83	---
4%D+10%E	---	15.24	2.84	---
4%D+20%E	---	15.31	2.7	1.4
4%D+20%E+0.1%T[c]	---	15.1	2.75	---
6%D+20%E	---	9.33	2.33	---

[a] 2,4,6-tris(dimethylaminomethyl) phenol and salicylic acid added as catalyst to speed up the reaction

[b] 0.1 Molar sodium carbonate buffer added.

[c] 0.1% polysorbate (Tween) 80 added.

RT = room temperature

Figure 1 is a log graph comparing the effect over time of exposure to 2% solutions of the sterilant in combination with different surface tension reducing agents upon the above described Bacillus pumilus populations. The effect of varying the temperature from room temperature to 37 degrees C. can also be seen.

Figure 2 is a log graph comparing the effect with time of exposing Bacillus pumilus to different concentrations of sterilant and surface tension reducing agent at a constant temperature of 37 degrees C. The effect of increasing the

alcohol content of the solution from 5 to 20 volume percent was pronounced when the concentration of sterilant was 2 volume percent, but little benefit was gained from increasing the concentration of sterilant from 4 to 6 volume percent at a constant 20 volume percent concentration of alcohol.

The log graph in Figure 3 compares the effect of 2 and 4 volume percent concentrations of sterilant at temperatures of 37 and 45 degrees C. upon populations of Bacillus pumilus. The best results are achieved by using a 4% solution at a temperature of 45 degrees C.

EXAMPLE 2

Four types of in vitro biological tests were performed to evaluate the effects of ethylene glycol diglycidyl ether upon cell culture. The grafts had an approximate outside diameter of 0.6 cm, an inside diameter of 0.4 cm, and a surface area of 3.2 cm sq/cm of length and had been packaged in 70 volume percent of ethanol. To remove the ethanol from the grafts, they were rinsed in 300 ml. of normal saline for a minimum of two minutes.

The test extract solutions used were prepared by exposing sections of graft to normal saline or cottonseed oil for a period of 120 hours at 37 degrees C. to collect the residual sterilant therefrom. Saline and cottonseed oil closely mimic the effect of bodily contact upon a device in their ability to remove residual sterilant from the test articles.

Test 1, a procedure designed to evaluate the blood compatibility of the sterilant (Blood Compatibility), assesses the degree of hemolysis and the clotting time of blood contacted with an extract test solution prepared with a saline extract. The extract was exposed to oxalated whole rabbit blood for one hour, after which the absorbance of the solution was measured spectroscopically for hemoglobin content caused by lysis of the red cells in the

blood. In addition, pieces of graft (Solid) that had been washed but not extracted were also tested using the above procedure. In a second test related to the effect of the sterilant upon blood, the saline extract containing residual sterilant was exposed to sheep plasma and visually observed to determine the effects on clotting time.

Test 2 was designed to assess the inhibitory effect of an aqueous extract of residual sterilant on the normal growth of cells in culture (%ICG) using the following procedure. Human fibroblast cells were added to a test solution containing an equivalent mixture of a normal saline extract (37° C/120 hours) of the test article and cell culture media and were incubated for 72 hours. The amount of cell growth during the incubation period was determined by calculating the protein concentration of the incubated extract and comparing it with the protein concentration of a negative control solution prepared by adding an equal amount of cells to a test solution containing an equal amount of normal saline and cell growth media. This method yields a quantitative assessment of cytotoxicity.

In Test 3, the agar overlay test (AO), cytotoxicity of diffusible components of the test solution through an agar layer was evaluated. In preparation for the test, human fibroblast cells were cultured in a petri dish to form a confluent monolayer. The cells were then overlayed with bacto agar, and the agar layer was allowed to solidify before 1.0 cc of the test solution was placed on the solidified agar. After 24 hours, the cells were examined under microscope (X100) for damage or lysis. The agar overlay test (AO) was also conducted substituting a section of solid graft (Solid) for the test solution.

In Test 4 the cytotoxic effect of the sterilant was tested using the Medium Eluate Method (MEM). A monolayer of human fibroblast cells was incubated in the presence of

a normal saline/cell growth medium extract of the graft for 24 hours. A negative control was prepared by incubating a monolayer of human fibroblast cells in growth medium in the place of the graft extract. The cells were then examined for evidence of damage of lysis under a microscope (100x). In the results in Table 2, the designation "pass" was given to samples in which cell destruction was not significantly greater than that found in the negative control.

Two additional in vivo biological tests were performed using the test solutions prepared as above described. to evaluate the systemic effect of the sterilant on animals. In Test 5 (Mouse Systemic Injection), the solution was injected into each of five mice intravenously (for normal saline) or intraperitoneally (for cottonseed oil) at a dosage of 1.0 ml./20g. of body weight. The animals were observed for 72 hours post injection for any adverse reactions, and the observations were compared against those of the negative control animals. In Test 6 (Rabbit Intracutaneous Irritation), the effect of the extract upon contact with the dermis of rabbits was evaluated. Ten test sites and five control sites on the backs of each of two rabbits were injected intracutaneously with 0.2 ml. of the test solution. The test sites were examined for signs of irritation visualized as erythema and/or edema at 24, 48 and 72 hours.

The results of the biological tests are summarized in Table 2 below.

TABLE 2

BIOLOGICAL EFFECTS OF ETHYLENE GLYCOL DIGLYCIDYL ETHER

Test Procedure	Extract	Test 1	Test 2
In vitro:			
%ICG	NS	PASS	PASS
MEM	NS	PASS	PASS
AO	NS	PASS	PASS
	Solid	PASS	PASS
Blood Compatibility	NS	PASS	---
	Solid	PASS	---
In vivo:			
Mouse Systemic	NS	PASS	PASS
	CSO	PASS	PASS
Rabbit Intracutaneous	NS	PASS	PASS
	CSO	PASS	PASS

NS = Normal saline
CSO = cottonseed oil

The designation "Pass" indicates that the results of the particular effect being tested (ie., the amount of lysed red blood cells) are not significantly worse for the test solution than for a negative control sample. From the test methods employed, the polyepoxy compound shows no signs of cytotoxicity or systemic toxicity and does not appear to be intracutaneously irritating or hemolytic.

EXAMPLE 3

In this study three polyepoxy sterilants were tested: glycerol polyglycidyl ether, polyglycerol polyglycidyl ether, and ethylene glycol diglycidyl ether. Samples collected from each of three bovine carotid glutaraldehyde fixed grafts stored in 30 volume percent ethanol were used in each of the following tests. The grafts were approximately 0.540 cm. in outside diameter, 0.443 cm. in inside diameter, 10 cm. in length, and had a surface area of approximately 3.09 cm. sq. per cm. of length. The results of the tests appear below in Table 3.

In Test 7 the cytotoxicity of diffusible components of the test solution through an agar layer (AO) was evaluated as in Test 3 above. For this study only a 2 cm. section of graft was evaluated. The section was removed from each test article, and the sections were rinsed separately in 100 ml. normal saline for 5 minutes with occasional flushing of the graft section by squeezing. The rinsing step was repeated 5 times prior to testing.

In Test 8 The inhibitory effect of a sterile water extract of residual sterilant on the normal growth of cells in culture (%ICG) was performed using the method of Test 2 above. For this study a 20 cm. section of graft removed from each test article was rinsed separately in 100 ml. of normal saline for 5 minutes with occasional flushing of the graft section by squeezing. The rinsing was repeated 5 times prior to testing. The test article was then extracted in sterile water at 37 degrees C. for approximately 120 hours, the extract was then filter sterilized, mixed with an equivalent volume of cell growth medium, and evaluated.

In Test 9, the method of Test 4 above (MEM) was followed. For this study an 8 cm section of graft was removed from each graft for extraction. The sections of graft were rinsed separately in 100 ml. of normal saline for 5 minutes with occasional flushing of the graft section by squeezing. This rinsing procedure was repeated 5 times. The sections of graft were then extracted in Complete Minimum Essential Medium (a cell growth medium) for approximately 24 hours prior to testing.

TABLE 3

BIOLOGICAL EFFECTS OF POLYEPOXY STERILANTS

		Sterilant		
		EGDE	DDE	GDE
Test Procedure	Extract	(Denacol® 810)	(Denacol® 512)	Denacol® 313
%ICG	Water	PASS	PASS	PASS
MEM	1xCMEM	PASS	PASS	PASS
AO	N/A	---	---	---

EDGE = ethylene glycol diglycidyl ether
DDE = diglycerol diglycidyl ether
GDE = glycerol diglycidyl ether
N/A = not applicable

As shown in Table 3 above, none of the samples in the %ICG and MEM procedures demonstrated signs of cytotoxicity. Results from the AO procedure were invalid because the cell monolayers in the test samples and the negative controls all failed to maintain confluency.

The present invention has been described in specific detail and in reference to its preferred embodiment; however, those skilled in the art will understand that modifications and changes can be made to the invention without departing from its spirit and scope.

WHAT IS CLAIMED IS:

1. A method for sterilizing a medical device comprising:

contacting the device with a sterilization effective and residually non-toxic amount of at least one polyepoxy compound so as to substantially reduce the microbial population thereon.

2. The method of claim 1 wherein the polyepoxy compound is selected from the group consisting of glycidyl ethers.

3. The method of claim 2 wherein the glycidyl ethers are selected from the group consisting of diglycidyl ether of glycol, polyglycidyl ether of polyol, diglycidyl ether of dicarboxylic acid and monoglycidyl ether of polyol.

4. The method of claim 3 wherein the glycidyl ethers are selected from the group consisting of ethylene glycol diglycidyl ether, diglycerol diglycidyl ether, and glycerol diglycidyl ether.

5. The method of claim 1 wherein the sterilization temperature is from about room temperature to 45 degrees Centigrade.

5. The method of claim 1 wherein the polyepoxy compound is in a solution at a concentration from about 2 to 4 percent by volume.

6. The method of claim 1 wherein the contacting time is from about 5 to 120 hours and the microbial population is reduced by at least 90 percent.

7. The method of claim 1 wherein the solution is aqueous and further comprises from about zero to 30 volume percent of at least one surface-tension reducing agent.

8. The method of claim 7 wherein the concentration of the surface-tension reducing agent is from about 5 to 15 volume percent.

9. The method of claim 7 wherein the surface tension reducing agent is a biocompatible aliphatic or aromatic alcohol.

10. The method of claim 9 wherein the alcohol is an aliphatic alcohol containing from 1 to about 5 carbon atoms.

11. The method of claim 10 wherein the alcohol is selected from the group consisting of ethanol and isopropanol.

12. The method of claim 9 wherein the alcohol is an aromatic alcohol.

13. The method of claim 12 wherein the aromatic alcohol is selected from the group consisting of benzyl alcohol, cresol, and carbinol.

14. The method of claim 7 wherein the surface tension-reducing agent is selected from the group consisting of anionic, cationic and nonionic surfactants and salts thereof.

15. The method of claim 14 wherein the salts are sodium and potassium salts.

16. A method for sterilizing a biological prosthetic device comprising:

contacting the device with a solution comprising a sterilization effective amount of at least one polyepoxy

compound so as to substantially reduce the microbial population thereon.

17. The method of claim 16 wherein the polyepoxy compound is selected from the group consisting of glycidyl ethers.

18. The method of claim 17 wherein the glycidyl ethers are selected from the group consisting of diglycidyl ether of glycol, polyglycidyl ether of polyol, diglycidyl ether of dicarboxylic acid and monoglycidyl ether of polyol.

19. The method of claim 18 wherein the glycidyl ethers are selected from the group consisting of ethylene glycol diglycidyl ether, diglycerol diglycidyl ether, and glycerol diglycidyl ether

20. The method of claim 16 wherein the temperature of the solution is from about room temperature to 45 degrees Centigrade.

21. The method of claim 16 wherein the concentration of the polyepoxy compound in the solution is from about 2 to 4 percent by volume.

22. The method of claim 16 wherein the contacting time is from about 5 to 120 hours and the microbial population is reduced by at least 90 percent.

23. The method of claim 16 wherein the solution is aqueous and further comprises from about zero to 30 volume percent of at least one surface-tension reducing agent.

24. The method of claim 23 wherein the concentration of the surface-tension reducing agent is from about 5 to 15 volume percent.

25. The method of claim 24 wherein the surface tension reducing agent is a biocompatible aliphatic or aromatic alcohol.

26. The method of claim 25 wherein the alcohol is an aliphatic alcohol containing from 1 to about 5 carbon atoms.

27. The method of claim 26 wherein the alcohol is selected from the group consisting of ethanol and isopropanol.

28. The method of claim 25 wherein the alcohol is an aromatic alcohol.

29. The method of claim 28 wherein the aromatic alcohol is selected from the group consisting of benzyl alcohol, cresol, and carbinol.

30. The method of claim 23 wherein the surface tension-reducing agent is selected from the group consisting of anionic, cationic and nonionic surfactants and salts thereof.

31. The method of claim 30 wherein the salts are sodium and potassium salts.

32. The method of claim 18 wherein the temperature of the solution is from about room temperature to 40 degrees Centigrade.

33. The method of claim 18 wherein the concentration of the polyepoxy compound in the solution is from about 2 to 4 percent by volume.

34. The method of claim 33 wherein the contacting time is from about 4 to 60 hours.

35. The method of claim 16 wherein the device is a biological vascular graft.

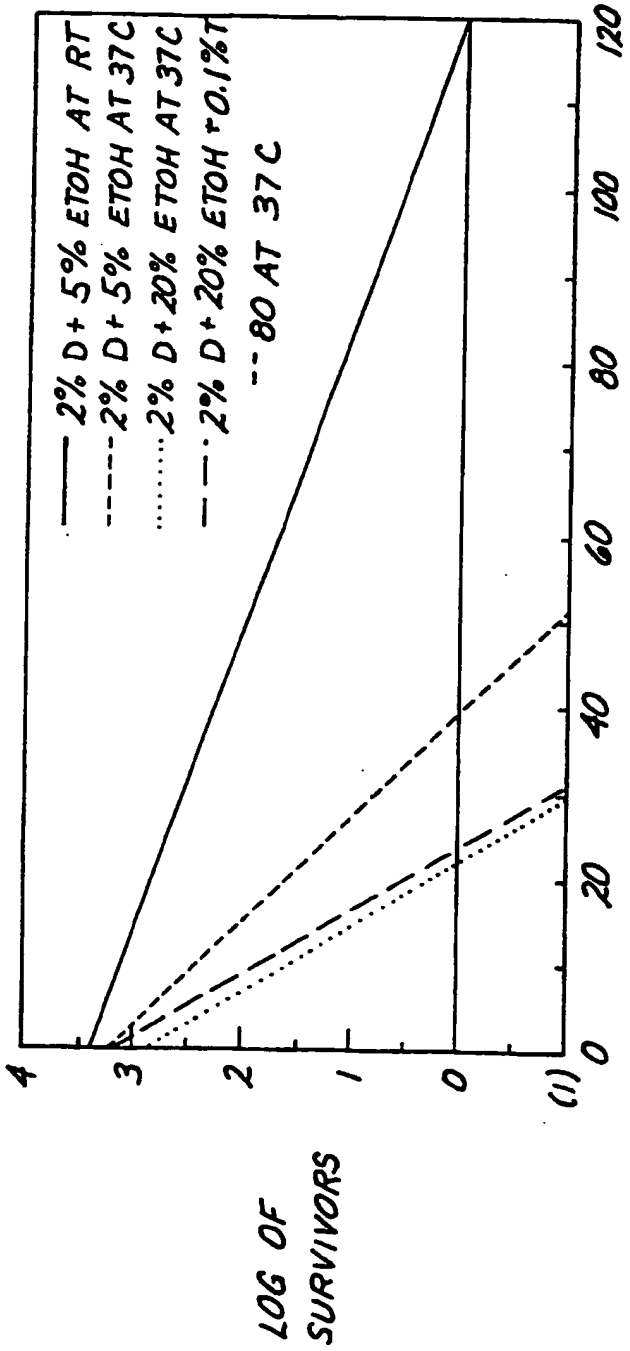

Fig. 1 BACILLUS PUMILUS VS. 2% DENACOL SOLUTIONS AT TEMPERATURE

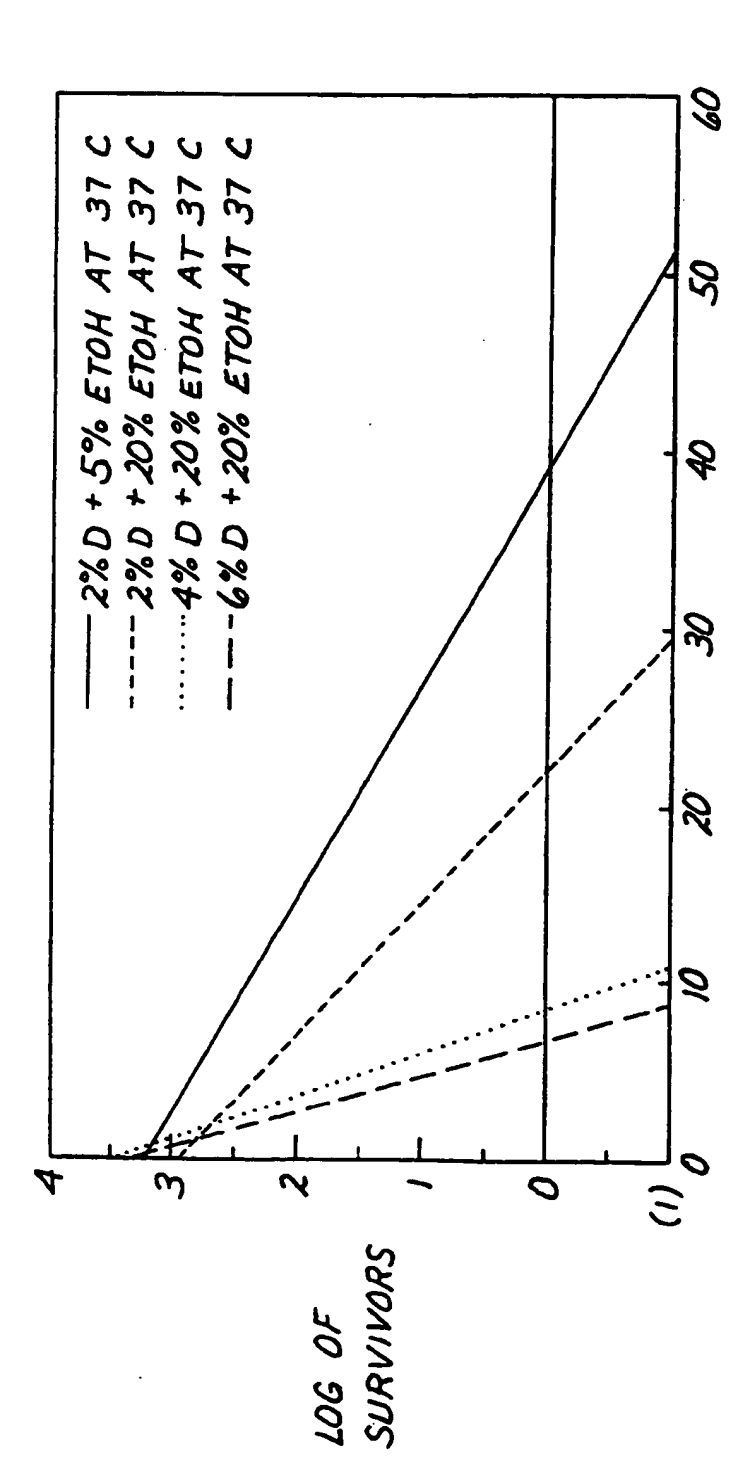

Fig. 2 BACILLUS PUMILUS VS. VARIOUS DENACOL SOLUTIONS AT TEMPERATURE

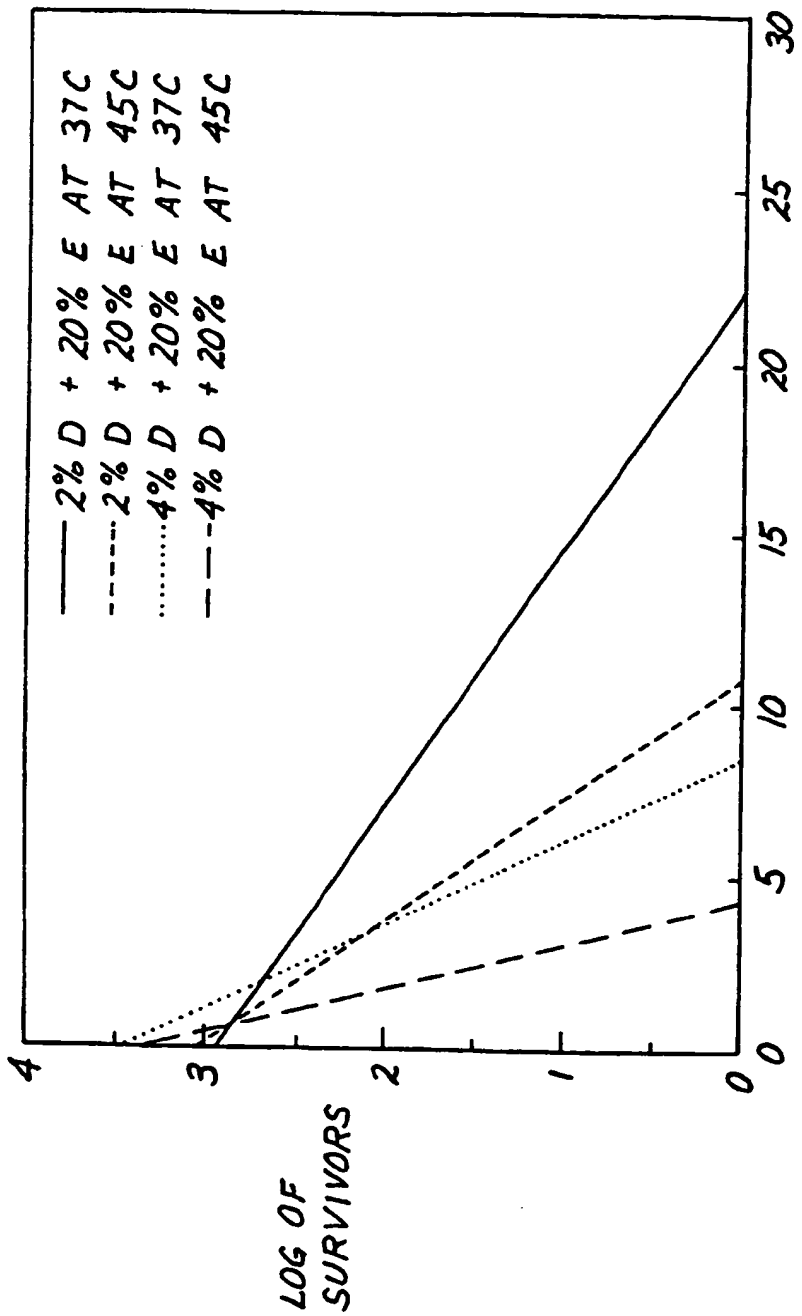

Fig. 3 BACILLUS PUMILUS VS. 2% & 4% DENACOL SOLUTIONS AT TEMPERATURE